MITOTIC EFFECTS OF PROLONGED IRRADIATION WITH LOW-INTENSITY GAMMA RAYS ON THE CHORTOPHAGA NEUROBLAST

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When cells are subjected to given doses of ionizing radiation delivered at very low rates, the detectable effects on mitotic activity, as measured at the end of the treatment period, resemble those obtained from smaller doses administered at higher intensities (Carlson, Snyder and Hollaender, 1949; Carlson, 1950). This is due, apparently, to the capacity of living cells to recover from the more immediate physical and chemical changes induced by irradiation even while treatment is in progress. At the end of a short treatment period the reduced mitotic activity will represent virtually all the potential mitotic effect produced, because little recovery from these initial effects will have occurred. At the end of a long period of irradiation, on the other hand, the decreased mitotic activity will be the result of only the physico-chemical changes produced in the later part of the treatment period, those induced earlier having undergone recovery before the period of observation.

The main purpose of the present study was to investigate the mitotic effect of prolonged low-intensity treatment and to determine whether a balance between effect and recovery would be established and maintained at about the same level over much of the extended periods during which treatment was in progress. If such a balance were established, the detectable mitotic effects would be approximately the same at the ends of a wide range of treatment periods for a given dosage rate.

Materials and Methods

Eggs of *Chortophaga viridifasciata* (DeGeer) were used in all experiments. Because the times of treatment ranged from two to six days, it was necessary to start with embryos sufficiently young so that at the end of treatment they would be at the optimum age (14 days at 26° C.) for studying the mitotic activity of the neuroblasts.

The apparatus used in this study to irradiate the grasshopper eggs with γ rays is shown in Figure 1. The source (B) consisted of activated cobalt-aluminum alloy in the form of a hollow cylinder 8 inches long and 2 inches in diameter with walls 0.03 inch thick. This was enclosed in a cylindrical lead pig (A), the walls and lid of which each measured 2 inches in thickness. A cylinder of polystyrene

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(C), bored from one end to a depth of 5½ inches with a ¾-inch bit, fitted snugly inside the source. Four polystyrene vessels (E), which were threaded so that they could be screwed to one another and to a polystyrene plug (D) held the eggs during treatment. The interior dimensions of each vessel, when screwed together, were approximately ½ inch in diameter and ¼ inch in depth. Treatment was begun by inserting the joined vessels and plug, vessels first, into the bore of the polystyrene cylinder (C). When in this position, the material to be treated was situated no more than one inch above or below the middle of the source, which extended at least three inches beyond the vessels at either end. Treatment was terminated by withdrawing the plug and attached vessels.

FIGURE 1. Gamma source and accessories. A, lead pig; B, cobalt-aluminum alloy source; C, polystyrene cylinder; D, polystyrene plug; E, polystyrene vessels; F, polystyrene vessel (enlarged) containing grasshopper eggs.

This type of source has several advantages (see Sheppard, 1949). The dosage rate can be determined to an accuracy of about 2%. The long half life of the cobalt (5.3 years) makes it possible to use a given source over a period of several months with very little decrease in the dosage rate. Because the source is cylindrical and its interior is filled with polystyrene, the radiation field is quite uniform throughout the space containing the biological material. Further, low-intensity treatment can be maintained at a constant level over long periods of time inside a protective lead container occupying less than a square foot of the floor.

Ideally, all the space in the specimen vessels not occupied by the biological material should contain water. Since the complete immersion of grasshopper eggs in water affects mitoses very adversely, presumably because of the reduced

exchange of oxygen and carbon dioxide between the egg and the surrounding medium, only enough moisture was placed in each vessel to keep the eggs from drying out. The pocket of air in each vessel is so small (about 0.05 cubic inch) that it should have no appreciable effect on the amount of radiation received by the eggs. The vessels were opened for a very brief period every 48 hours in the 3.4 r/hour experiment and twice daily in the 0.80 r/hour experiments during the treatment period to prevent accumulation of excessive amounts of carbon dioxide and depletion of oxygen in the vessel containing the eggs. In all experiments, irradiated and control embryos were from the same egg pods and were handled identically except for the presence or absence of the activated cobalt-aluminum cylinder in the pig.

The source was calibrated by substituting for the plug and vessels in the polystyrene bore a thimble chamber of the type designed by Darden and Sheppard (1951) and comparing the intensity of ionization with that measured when the same chamber was exposed to a radium source of known mass.

At the end of the irradiation period, which lasted for 2, 4 or 6 days, control and treated eggs were removed from the vessels and made into hanging-drop preparations by the method described previously (Carlson and Hollaender, 1944; Carlson, 1946).

Except in the final experiment, in which the numbers of middle and late prophases were also recorded, mitotic activities of the treated and control embryos were determined by counting the mid-mitotic [3] neuroblasts from the first maxillary through the first abdominal segments at 22-minute intervals. The average time required by the neuroblast to pass through mid-mitosis is about 22 minutes; therefore, the total of a number of such counts approximates the total number of cells undergoing mitosis within the period of time involved. Information on the number of counts made, the time after treatment of the initial count, the dosage rate, and the time of treatment, which differed from one experiment to another, is included with the description of the individual experiments.

Observations and Interpretation

Dosage rate of 3.4 r/hour

The mitotic effects of treatment for 6 days (143–145 hours) at a dosage rate of 3.4 r/hour are shown in Figure 2. The first count was made at an average of 16 minutes [4] after removal of the embryos from the source; subsequent counts were made at 22-minute intervals. Except for the second, or 38-minute, counting period, the number of mid-mitotic cells is reduced by this treatment to approximately 0.4 of normal. This is in striking contrast to what would have been obtained, if the same total dose—490 r—had been delivered in a few minutes instead of 6 days. Then the mitotic activity of the irradiated cells would have been

[3] This refers to cells in prometaphase, metaphase, or anaphase, *i.e.*, those between the breakdown of the nuclear membrane at the end of prophase and the loss of distinctness in the appearance of the chromosomes that marks the advent of telophase.

[4] The times after irradiation at which the initial counts were made differ from experiment to experiment, because we did not recognize the importance of an immediate first count until the early experiments had been completed.

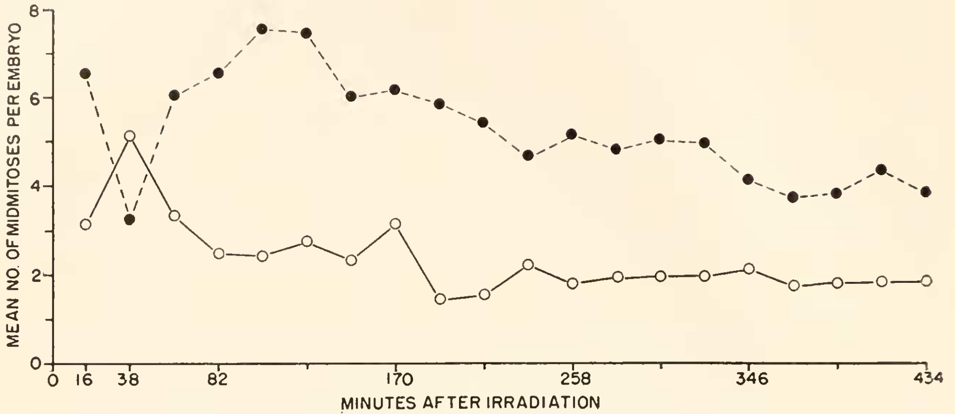

FIGURE 2. Dosage rate, 3.4 r/hour. Duration of treatment, 143–145 hours; dose, 489.6 r. ○, irradiated cells; ●, control cells

reduced to zero within an hour after treatment and would have remained at that level for about 5 hours. This demonstrates clearly the capacity of cells to undergo recovery from mitotic effects of radiation during the period of treatment.

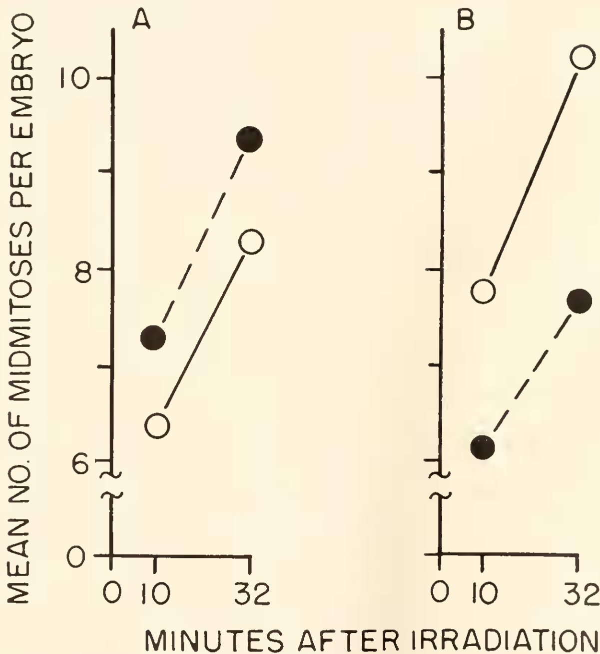

FIGURE 3. Dosage rate, 0.80 r/hour. A, duration of treatment, 44–51 hours; approximate dose, 35–41 r. B, duration of treatment, 92–99 hours; approximate dose, 74–79 r. ○, irradiated cells; ●, control cells

TABLE I

Summary of data and biometrical analysis

Dose rate (r/hr.)	Duration of irradiation (hrs.)	Approximate dose (r)	No. of embryos		No. of counting periods	Stages counted	Per cent level of significance		Least significant difference (5% level) between treated and control embryos for any one counting period*	Standard error of a single count*	Degrees of freedom for error
			Irradiated	Control			Between treated and control counts averaged over all counting periods	For interaction between treatment and counting period			
3.4	143–145	490	16	16	20	Mid-mitosis	<0.1	<0.1	0.46	0.67	562
0.80	44–51	35–41	31	31	2	Mid-mitosis	20	>50	0.30	0.60	58
	92–99	74–79	20	20	2	Mid-mitosis	3.6	43	0.42	0.64	38
	144	115	11	11	16	Mid-mitosis	>50	<0.1	0.54	0.65	300
						Middle prophase	42	>50	0.76	0.81	73
	144	115	15	14	4	Late prophase	<0.1	9.7	0.53	0.68	73
						Mid-mitosis	>50	18	0.60	0.89	77

* These figures should not be compared with the mean counts shown on the graphs, but with their approximate square roots. See text footnote 5.

The statistical analysis of the 3.4 r/hour data[5] summarized in Table I shows that the average numbers of mid-mitotic cells in treated and control embryos averaged for the whole counting period differ significantly at the 0.1% level of probability. Interaction is indicated by the fact that differences between treated and control embryos vary significantly at the 0.1% level from count to count.

Dosage rate of 0.80 r/hour

After two-day exposures at this dosage rate, mid-mitotic counts at 10 and 32 minutes after irradiation (Fig. 3A) were not significantly different from those of the control embryos, with respect to the counts averaged over all counting periods or the interaction (Table I). After four-day exposures, however, the mid-mitotic counts at both 10 and 32 minutes after irradiation were higher than in the controls (Fig. 3B). Biometrical analysis indicates a significant difference at the 3.6% level for the counts averaged over all counting periods and for the second counts alone at the 5% level, but not for the first count or for interaction (Table I).

Some preliminary 6-day exposures at this dosage rate also gave initial mid-mitotic counts that were distinctly higher than those of the control embryos. This unexpected result, it was reasoned, could have resulted from any of three factors. (1) Prolonged low-intensity radiation might have a stimulating effect on mitosis. By this we mean that a more rapid passage of cells through the stages of the mitotic cycle between the end of anaphase and the beginning of prometaphase, namely, telophase, interphase, or prophase, might throw a proportionally greater number of the cells into mid-mitosis. (2) The progress of cells through the stage being counted, namely, mid-mitosis, might have been retarded, so that the same cell was included in successive counts, thus giving a false picture of increased mitotic activity. (3) Cells might have been retarded in late prophase, accumulating there in abnormally large numbers, and then progressing simultaneously into mid-mitosis after removal from the field of radiation.

With these possibilities in mind an experiment was set up to determine whether or not this initial "stimulating" effect was the result of counting the same mid-mitotic cell twice in successive counts. Treatment was at the rate of 0.80 r/hour for 6 days (total dose, 115.2 r). Sixteen successive counts were made at 22-minute intervals beginning 22 minutes after the end of radiation. During each observation period, neuroblasts in mid-mitosis were not only counted but also the exact stage and the location of each cell were recorded. From these data it was possible to correct for all cases in which a cell was counted twice by eliminating the second record. The only counting period for which this correction averaged more than 0.3 count per embryo was the second. Corrections for that period reduced the average count from 13.3 to 11.2 per irradiated embryo and from 6.0 to 4.55 per control embryo. The corrected results are used in Figure 4.

Biometrical analysis (Table I) indicates that there is no significant difference in the mitotic activity of the treated and control embryos for the period of observation taken as a whole. Interaction, however, is significant at the 0.1% level. The means of the 22- and 44-minute counts (Fig. 4) of the treated cells are sig-

[5] All analyses were based on the square root of counts, because this transformation makes variances approximately uniform.

nificantly higher than those of the untreated cells while the means of the 110-, 132-, and 154-minute counts of the treated cells are significantly lower than those of the control cells at the 5% level. The possibility that the higher initial number of mid-mitotic cells in the treated than in the control embryos resulting from counting the same cell twice is, therefore, eliminated.

To determine whether the high initial number of mid-mitotic cells results from simultaneous recovery and mitotic progression of an abnormally large number of cells retarded at middle and late prophase by the irradiation, an experiment was

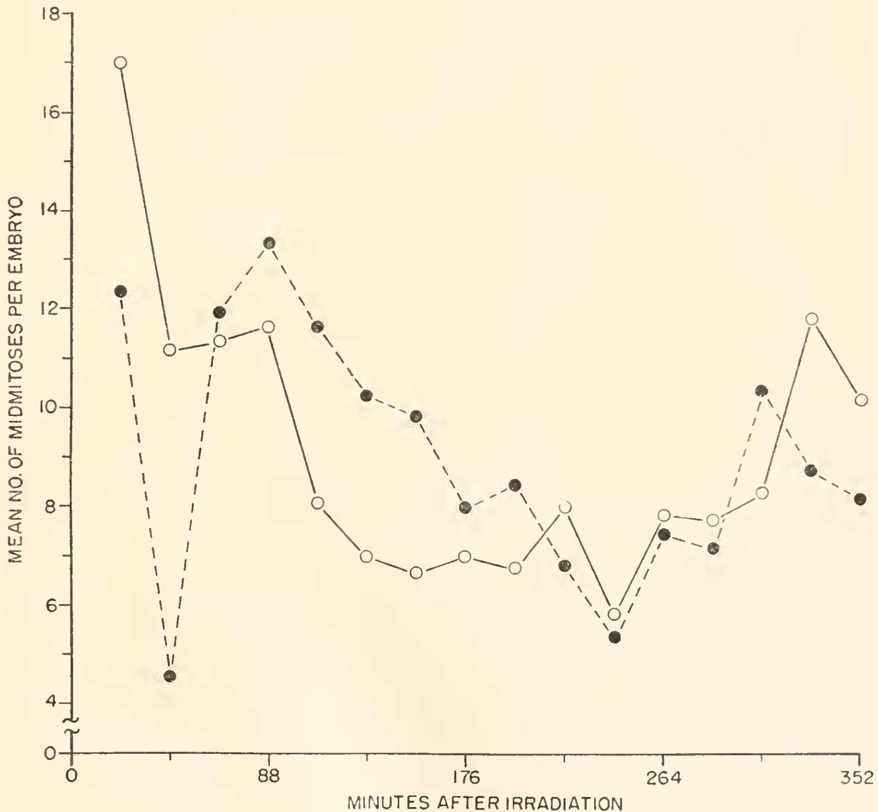

FIGURE 4. Dosage rate, 0.80 r/hour. Duration of treatment, 144 hours; dose, 115 r. ○, irradiated cells; ●, control cells

run in which the counts were made of middle prophases, late prophases and mid-mitoses. The first count was made as soon after irradiation as the material could be prepared for observation, *i.e.*, 4.5–6.0 minutes after removal from the radiation field. Three additional counts were made 22, 44, and 66 minutes after the end of radiation.

The results are shown graphically in Figure 5. The 22-minute mid-mitotic count, like that in the preceding experiment, is considerably higher than the corresponding control count. The difference is significant at the 8.2% level (Table II).

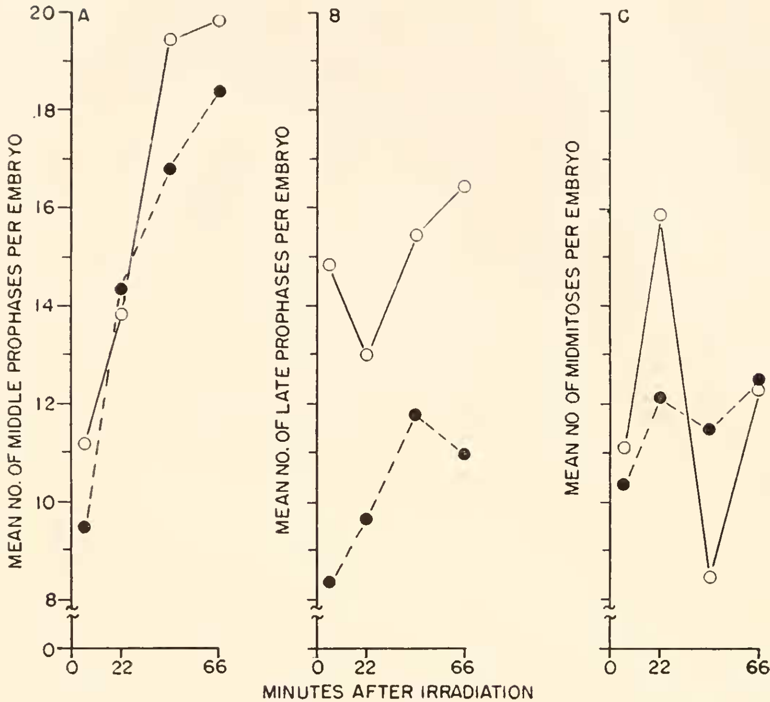

FIGURE 5. Dosage rate, 0.80 r/hour. Duration of treatment, 144 hours; approximate dose, 115 r. A, middle prophases; B, late prophases; C, mid-mitoses. ○, irradiated cells; ●, control cells

The immediate (4.5- to 6.0-), 44-, and 66-minute counts, however, are not significantly different in the irradiated and control embryos. Most of the cells in mid-mitosis at the time of the 22-minute count were in late prophase at the time of the preceding count (see mitotic time schedule, Carlson and Hollaender, 1948). Examination of the initial count of late prophase cells shows a significantly greater number of irradiated than of control late prophases at the 0.1% level. This supports the second possibility mentioned earlier that the abnormally high early

TABLE II

Comparison of treated and control counts of middle prophases, late prophases, and mid-mitoses for four counting periods following 144 hours of irradiation at 0.80 r/hour

Minutes after irradiation	Per cent level of significance between irradiated and control embryos		
	Middle prophase	Late prophase	Mid-mitosis
4.5–6.0	55	<0.1	65
22	88	5.5	8.2
44	46	8.5	21
66	64	0.5	87

mid-mitotic count results from the gradual accumulation of many cells in late prophase during the long irradiation period. That the inhibitory effect of the irradiation on late prophase cells persists in some measure even as late as 44 and 66 minutes after treatment is evidenced by the significantly greater numbers (at the 8.5 and 0.5% levels, respectively) of late prophases at these counting periods.

The irradiated middle prophases are not significantly different from the controls with respect to averages over all counting periods, interaction, or any of the four counts.

It seems worth while to point out that the mean counts (Fig. 5) in treated embryos are higher than those of the control embryos (1) for all but one of the middle prophase counting periods, (2) for all of the late prophase counting periods, and (3) for two of the four mid-mitotic counting periods. Even though only three of the higher ones are *significantly* higher at the 5.5 level, the fact that three-fourths of all of them are higher emphasizes the tendency of neuroblasts to accumulate in certain parts of the mitotic cycle when subjected to very low doses of radiation.

These results demonstrate again what has become increasingly evident over the years during which the mitotic effects of irradiation have been studied, namely, that radiation can retard but not stimulate the mitotic progress of cells. It appears that any increase in the number of cells in a given stage of mitosis soon after treatment can be interpreted to result from either retardation of mitotic progress within that stage resulting in an accumulation of cells, or entry into that stage in a brief period of time of an abnormally large number of cells that accumulated in a preceding stage as a result of mitotic retardation.

Further substantiation is also given to the transitory nature of the physicochemical radiation-induced effects that may lead ultimately to mitotic retardation. The results of earlier ultraviolet (Carlson and Hollaender, 1945) and γ-ray (Carlson, Snyder and Hollaender, 1949) studies indicated that the smaller mitotic effect of a large dose of low, as compared with high, intensity irradiation was probably due to less opportunity for interaction of primary radiation effects. In the present study the dosage rate has been so low that the chance of interaction has probably been virtually eliminated.

The authors are greatly indebted to Dr. C. W. Sheppard for designing and calibrating the γ-radiation source for special use in this project, and to Dr. A. W. Kimball for the biometrical analysis of the data.

Summary

1. Prolonged treatment of *Chortophaga* neuroblasts with low-intensity γ radiation reduces mitotic activity much less than a comparable dose given at high intensity.

2. Treatment for 6 days at 3.4 r/hour reduced the mid-mitotic count to about 40% of normal for a period of about 7 hours following treatment. (The same dose administered in a few minutes would have reduced the mid-mitotic count to zero for about 5 hours beginning within an hour of the end of treatment.)

3. Continuous irradiation at 0.80 r/hour (1) failed to produce a significant mid-mitotic effect at the end of two days, (2) produced a significant *increase* in

the number of mid-mitoses through the second counting period at the end of four days, and (3) produced a significant *increase* in the number of mid-mitoses through the second counting period after 6 days of treatment.

4. The radiation-induced increase in the number of mid-mitoses immediately following four and six days of treatment is shown to result, not from a stimulating effect of the radiation on mitotic activity, but from the simultaneous progression into mid-mitosis of neuroblasts that have accumulated in late prophase as a result of the inhibiting effect of the radiation on this stage.

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